Renewable and Sustainable Energy Reviews 30 (2014) 1035–1046

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Microalgal biofuels: Flexible bioenergies for sustainable development

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ARTICLE INFO

Article history:
Received 30 July 2012
Received in revised form
2 September 2013
Accepted 11 November 2013
Available online 2 December 2013

Keywords:
Microalgae
Biofuels
Biomass
Sustainability

ABSTRACT

To confront energy shortage, global warming and climate changes, biofuels derived from biomass have received increasing attention from the industry, academia and governments. Of the potential sources of biofuels a most promising one is the simple photosynthetic microalgae, which can be grown in open ponds, photobioreactors and fermenters. The advantages to produce biofuels from microalgae include easy adaption to environmental conditions, high photosynthesis efficiency, high lipid content and noncompetition for farmlands. Nonetheless, the real hallmark of microalgae is the fact that these microscopic organisms can provide the biomass feedstock for the flexible production of several different types of renewable and sustainable biofuels such as biodiesel, bioethanol, biogas, biohydrogen among others via thermochemical and biochemical conversion processes. Amazingly, from a sustainability perspective the integrated algal biofuels production, where biodiesel, bioethanol and biogas are continuously produced from one biomass source, can evidently lead to an increase in the energetic productivity of the microalgal biomass, thus improving the economics of this algal biorefinery approach. Developments in several areas, such as genetic and metabolic engineering, are expected to further improve the costeffectiveness of the biofuels from microalgae in an environmentally sustainable manner.

Contents

1. Introduction

Today more than ever before, energy shortage, global warming and climate changes are distracting people's focus of production development towards renewable and sustainable energy. One of the main existent problems is that the high number of on-road diesel vehicles implies that the emissions from the engines can

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http://dx.doi.org/10.1016/j.rser.2013.11.003

significantly contribute to the atmospheric levels of the most important greenhouse gas, CO_2 and other urban pollutants, such as CO, NO_x, unburned hydrocarbons, particulate matters and aromatics [1,2]. In EU the transport sector, which accounts for more than one-third of its total energy demand, uses 98% of fossil oil to produce fuels for vehicles [3]. The UK Energy Research Centre even made a conclusion that the well-available conventional resources will be exhausted between 2020 and 2030 when analyzing global oil depletion [4]. However, new oil and gas reserves, such as oil reserves lying under the Arctic ice [5], have constantly been found. Undoubtedly, the potential oil and gas refining will increase fossil fuel reserves [1], thus causing the risks of the exponential rise of greenhouse effect. Carbon dioxide emissions from fossil fuel combustion witnessed an increase of 41% between 1990 and 2008 [6]. The global warming and climate changes highly related to CO_2 emissions can have significant effects on the environment and is considered as a main factor to raise the frequency and intensity of natural catastrophes and create climate-related hazards [1], such as a rise in sea level, heat wave attack, frequent intense storms and heavy precipitation events, and drought intensification [7]. To confront the issues of energy shortage, global warming and climate change, renewable and alternative energy sources have received a lot of attention and interest for biofuels production in an attempt to search for sustainable development.

Biofuels, which can be used as a substitute for petroleum fuels [8], are derived from biomass through some physical and chemical processes [9]. Biomass is any organic material which has stored solar energy in the form of chemical energy. As a biofuel source, biomass may include wood, wood waste, straw, manure, sugarcane, energy plants, and many other byproducts from a variety of agricultural processes. Although coal, crude oil, natural gas and other non-renewable energy sources have their origin in ancient carbon fixation, they are not regarded as renewable energy types by the commonly accepted definition, due to the fact that they contain carbon which has been "out" of the carbon cycle for tens of thousands of years [10]. Biofuels can be categorized into three types based on the biomass sources: the first generation biofuels; the second generation biofuels and the third generation biofuels. The most popular types of the first or second generation biofuels are biodiesel from, for example, palm, canola and jatropha [11,12], and bio-ethanol from crops, such as corn starch and sugar cane [13–15]. In addition, recently biomass-derived butanol and a new class of oxygenated biofuel known as furan have been developed due to their higher energy density [16,17]. Biofuels production can increase the employment opportunities for people in rural areas especially in developing countries, and it can also decrease a country's reliance on crude oil imports [18]. However, the growth of these food or non-food crops for bioefuels production will compete for limited arable farmlands, which should be used to grow crops for food production. And thus it might increase food prices, and subsequently affect the poor, although in some areas it can increase income for farmers as an additional source [19]. In an effort to potentially solve most of the concerns that the first- and second generation fuels will confront to [20], algal fuels, also called the third generation biofuels [21,22], have gained a lot of interest in recent years [23–30].

Microalgae are unicellular or multi-cellular photosynthetic microorganisms, which grow in aquatic environments and convert CO_2, water and sunlight through photosynthesis to produce lipids, carbohydrates and proteins in large amounts [31]. They are categorized into two main classes: prokaryotic and eukaryotic microalgae. Examples of prokaryotic species are cyanobacteria (*Chloroxybacteria*), while eukaryotic microalgae are, for example, green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophta*) [32]. According to Richmond [33], more than 50,000 microalgae species exist, but only about 30,000 species have been studied and analyzed until now. Microalgae do not have real roots, stems, leaves and embryos, although they were once considered to be aquatic plants. They may be grown in shallow lagoons, raceway ponds, closed ponds, photobioreactors, fermenters and sea-based systems.

The idea of biofuels production from microalgae is not new, but currently it has received the keenest interests in an effort to combat global climate changes. Most of the studies have been focused on the following aspects: (1) microalgae culture system, including raceway, photobioreactor (PBR) and fermenter; (2) collection, screening and classification of microalgae, (3) molecular biology and genetic engineering; and (4) system analysis and resource assessment. The strengths of microalgae-based biofuels as the third generation biofuels are many. These species are known to produce considerably greater amounts of biomass and lipids, can be cultivated without occupying farmlands, do not compete with food crops, require less energy than other feedstock during conversion process, and can be supplied by salt and wastewater to provide the nutrients and CO_2-rich flue gas as carbon source [1].

This paper presents a brief review of the current knowledge on microalgae cultivation technology, the main biofuel products (e.g., biodiesel, bioethanol, biogas and biohydrogen) and the potential of the integration of algal biodiesel, bioethanol and biogas production in a biorefinery approach to search for a better understanding of microalgae-based biofuels production and path forward for research and commercialization. We hope that this systematic review will provide a valuable guideline to researchers, policy makers, governments and potential business traders in an effort to prosper the microalgae biofuels industry to become more feasible and economic in a sustainable manner.

2. Microalgae cultivation technology

Microalgae with the composition of $CH_{1.7}O_{0.4}N_{0.15}P_{0.0094}$ [34] are simple photosynthetic organisms living in aquatic environments, where they can convert CO_2 and H_2O to biomass using sunlight [31]. Factors influencing the microalgae growth include [35]: abiotic factors, such as light intensity and quantity, temperature, O_2, CO_2, pH, salinity and nutrients (N, P, K, etc.); biotic factors, such as bacteria, fungi, viruses and competition for abiotic matters by other microalgae species; operational factors, such as mixing and stirring degree, width and depth, dilution rate, harvest frequency and addition of bicarbonate. The cultivation technologies being pursued to produce microalgae for biofuels generation mainly include open ponds, photobioreactors and fermenters.

Open ponds [36–39] are the oldest and simplest systems for microalgae cultivation and are usually established as shallow ponds with walls to grow microalgae outdoors. Water and nutrients microalgae need can be easily added or supplied by runoff water from nearby land areas [35] or by wastewaters from industry and/or community and CO_2 by combustion gas from firing plant [1]. When the systems are operated, water, nutrients and CO_2 are continuously fed into the ponds, and biomass is harvested at the other end. The two main prototypes in practice are raceway ponds and circular ponds. The former ones are widely used for the large-scale commercial production of *Spirulina* and *Dunaliella*, while the latter ones are mainly utilized for the cultivation of *Chlorella* in Asian countries. With respect to culture mixing, the construction designs are variable. In practice, paddle wheels are installed in raceway ponds to agitate the culture, while circular ponds use a centrally located rotating pivot for culture mixture.

Photobioreactors (PBRs) are widely used since the use of them can overcome some problems that open ponds have, such as

potential pollution, susceptibility to environmental conditions, water loss by evaporation, suitability for limited strains and the occupancy of large land areas. Usually, PBRs are different types of tanks or closed systems where microalgae are cultivated [22]. PBRs are closed systems with transparent or translucent materials, such as plastic and glass. Similarly, a pond covered with a greenhouse can be regarded as a PBR [35]. They can be provided with indoor artificial light via light collection and distribution systems or use outdoor sunlight directly. A stream of sterilized water containing all necessary nutrients including CO_2 should be consistently introduced into the system during microalgae growth. Along with the growth of microalgal biomass, excessive culture overflows and is harvested in a continuous manner. Until now there are several types of PBRs, such as tubular PBRs [40], combined bubble column and inclined tubular PBRs [41,42], helical PBRs [43] and flat plate PBRs [43].

Fermenters [44–46] for microalgae cultivation have been receiving increased attention at present. Although most microalgae species grow phototrophically, some can use organic substrates (glucose, acetate, fructose, citrate, etc.) as the sole carbon and energy source [47,48]. Compared to the phototropic production of microalgae, heterotrophic growth has several strengths, including preexisting fermentation technology knowledge base, the high degree of process control, no light required, climatic condition independence and lower harvest costs [49]. Fermenters are accessible in a wide range sizes from 1 l to 500,000 l. Sufficient oxygen is required for the metabolism of microalgae and catabolism of substrates since O_2 contents will reduce during fermentation. Thus, O_2 is the sole biggest limitation factor preventing high growth rate [50]. Usually, fermentation cultivation can increase the cell density and total lipid contents, compared to phototrophic mode [51–53].

The main differences among open ponds, photobioreactors and fermenters are summarized in Table 1.

Offshore cultivation systems [58,59] are historically used to grow macroalgae, such as seaweed. Recently sea-based systems, either open or closed, have been designed to cultivate microalgae as well. For example, the National Aeronautics and Space Administration (NASA) is inventing a closed system called OMEGA to grow algae using plastic bags at an open ocean. Generally the techniques, which are often used in shallow and protected coastal areas, can be labor intensive and integrated with fish cultivation. The main advantage lies in land resource saving and the fact that freshwater is not necessary since microalgae can be supplied by the rich amount of seawater. Nevertheless, offshore cultivation systems have a high risk of failure since they are susceptible to weather conditions, ocean waves and tides [1]. Thus, more researches are still required in an attempt to improve their efficiency, stability and security.

3. Microalgal biofuel options

Microalgae contain several constituents, mainly including lipids (7–23%), carbohydrates (5–23%), proteins (6–52%) and some fat [60]. Thus, microalgae have been investigated as a versatile biofuel feedstock for the production of biodiesel, bioethanol, biogas,

Table 1
Characteristics comparison of open ponds, photobioreactors and fermenters.[a]

Parameter	Open pond	Photobioreactor	Fermenter
Land requirement	High	Variable	Low
Water loss	Very high, may also cause salt precipitation [54,55]	Low [54,55], and it may be high if water spray is used for cooling	Low
Hydrodynamic stress on algae	Very low	Low–high	Unknown
Gas transfer control	Low	High	High
CO_2 loss	High, depending on pond depth [54,55]	Low [54,55]	No CO_2 is required
O_2 inhibition	Usually low enough because of continuous spontaneous outgassing	High (O_2 must be removed to prevent photosynthesis inhibition)	O_2 supply should be sufficient
Temperature	Highly variable [54,55]	Cooling often required [54,55]	Should be controlled to some special level [45]
Startup	6–8 weeks [54,55]	2–4 weeks [54,55]	2–4 weeks [56]
Construction costs	High—US $100,000 per hectare [54,55]	Very high—US $1,000,000 per hectare: PBR plus supporting systems [54,55]	Low
Operating costs	Low—paddle wheel, CO_2 addition [54,55]	Very high—CO_2 addition, Ph-control, oxygen removal, cooling, cleaning, maintenance [54,55]	Very high—oxygen addition, cleaning, sterilization, maintenance
Limiting factor for growth	Light	Light	O_2
Control over parameters	Low	Medium	Very high
Technology base	Readily available	Under development	Readily available
Risk of pollution	High	Medium	Low
Pollution control	Difficult	Easy	Easy
Species control	Difficult	Easy	Easy
Weather dependence	High—light intensity, temperature, rainfall [54,55]	Medium—light intensity, cooling required [54,55]	Low
Maintenance	Easy	Hard	Hard
Ease of cleaning	Easy	Hard	Hard
Susceptibility to overheating	Low	High	Unknown
Susceptibility to excessive O_2 levels	Low	High	Unknown
Cell density in culture	Low—between 0.1 and 0.5 g L^{-1} [54,55]	Medium—between 2 and 8 g L^{-1} [54,55]	High—e.g., 15.5 g L^{-1} [57], 16.8 g L^{-1} [45], 80–110 g L^{-1} [56]
Light-induced products (pigments, chlorophyll, etc.)	No impact	No impact	Reduced
Surface area-to-volume ratio	High	Very high	Not applicable
Applicability to different species	Low	High	Low
Ease of scale-up	High	Variable (bubble column and tubular PBRs are easy)	High

[a] The main information is adapted from [48,35], while other additional information is cited from the references as shown in the Table.

bio-hydrogen and many other fuel types [32] via thermochemical and biochemical methods. The flexible bioenergies production from microalgae for sustainable development is theoretically viable as shown in Fig. 1.

3.1. Biodiesel

Biodiesel, typically produced from oil-plants including food crops, has received a lot of concerns about the sustainability of this practice [8,31,61]. Microalgae as an alternative to conventional crops, such as sunflower and rapeseed, can produce more oil and consume less space [62], as shown in Table 2. Microalgal biodiesel contains no sulfur and can replace diesel in today's cars with little or none modifications of vehicle engines [35], while, on the other hand, the use of it can decrease the emissions of particulate matters, CO, hydrocarbons, and SO_x [63]. Compared to biodiesel derived from land-based crops, producing a substantial amount of biodiesel from microalgae has been considered as the most efficient way to make biodiesel sustainable. The renewability of the microalgal–biodiesel–carbon dioxide cycle is justified to be positive and any increase in algal lipid content will contribute to the improvement of the cycle efficiency.

Biodiesel is a mixture of fatty acid alkyl esters produced by a transesterification process, where triglycerides react with methanol or ethanol, a mono-alcohol [23,65,66]. Processes for biodiesel production from microalgae and from food and non-food crops are similar [67]. For biodiesel production, two steps are necessary: algal oil extraction and transesterification. During algal oil extraction, lipids and fatty acids have to be extracted from the biomass using a solvent, such as hexane, ethanol (96%), or a hexane–ethanol mixture (96%), with the possibility that up to 98% of purified fatty acids can be obtained [33]. Extraction methods can include expeller press, ultrasonic-assisted extraction, hexane solvent method, soxhlet extraction and supercritical fluid extraction (http://www.oilgae.com). The most widely used extraction method is soxhlet extraction using hexane as a solvent with an extraction time of 4 h, followed by oil separation from extracts by distillation process [67]. Cravotto et al. [68] introduced a new method called high-intensity ultrasound and/or microwave extraction, which could greatly improve oil extraction with higher efficiency compared to conventional methods. They recommended that this was the best method to extract oil from marine microalgae *Crypthecodinium cohnii*, as the disruption of the tough algal cell wall could improve the extraction yield from 4.8% to 25.9% in the present of soxhlet.

Following extraction, transesterification is a process to convert the algal oil to esters, the final fuel type. During this procedure, alkaline or acidic, homogenous or heterogenous chemical catalysts can be used [69,70]. Transesterification is a reaction with three reversible steps in series, including triglycerides conversion to diglycerides, then to monoglycerides, and finally to esters (biodiesel) and glycerol (by-product). The overall biodiesel transesterification reaction is described in Fig. 2. One molecule of each triglyceride in the algal oil reacts with three molecules of methanol to produce three molecules of methyl esters, the biodiesel product, and one molecule of glycerol.

Many researches on microalgal biodiesel production have been conducted, since microalgae are readily accessible and easily cultivated [24,25,57,71–73]. Plata et al. [74] found that more than 90% of biodiesel yield from *Chorella vulgaris* can be achieved with methanol to oil molar ratio at 14, 0.42 wt% NaOH, reaction temperature at 43 °C and reaction time at 90 min. Al_2O_3 supported CaO and MgO catalysts were recently initiatively tested in transesterification of lipid in *Nannochloropsis oculata* and 97.5% of biodiesel yield was attained under reaction temperature at 50 °C, catalyst loading at 2 wt%, methanol to lipid molar ratio at 30:1 and reaction time at 4 h [75]. Nevertheless, large-scale commercialization of algae-based biodiesel production does not appear until now, since the main obstacle is its high production costs, for instance, from the requirement of the high-oil-yielding algae strains and effective large-scale facilities [42,76]. The economics of the technology eventually depends on the government subsidies and the future price of oil, in addition to optimized biomass yields [77]. However, along with the development of biotechnology and the increase of crude oil prices, algal biodiesel will outcompete all other fuels, and well-funded R&D projects and policy support can make this scenario a reality.

3.2. Bioethanol

Bioethanol, a biofuel to replace the fossil-derived petrol [78], is produced by fermenting a variety of sugars derived from the hydrolysis of starch from, for instance, sorghum, corn and sugarcane. However, sugarcane ethanol is not cost effective, while corn ethanol will also cause the competition with food production and impacts on food availability. In this situation, bioethanol from lignocellulosic feedstock is being developed [79]. Lignocellulosic feedstocks include woody sources such as aspen, energy crops such as switchgrass, agricultural wastes such as corn stover [80],

Table 2
Yields of bio-oils produced from a variety of crops [64].

Substance	Gallons of oil per acre per year
Corn	15
Soybeans	48
Sunflower	102
Rapeseed (canola)	127
Oil palm	635
Microalgae	
Based on actual biomass yields	1850
Theoretical laboratory yields	5000–15,000

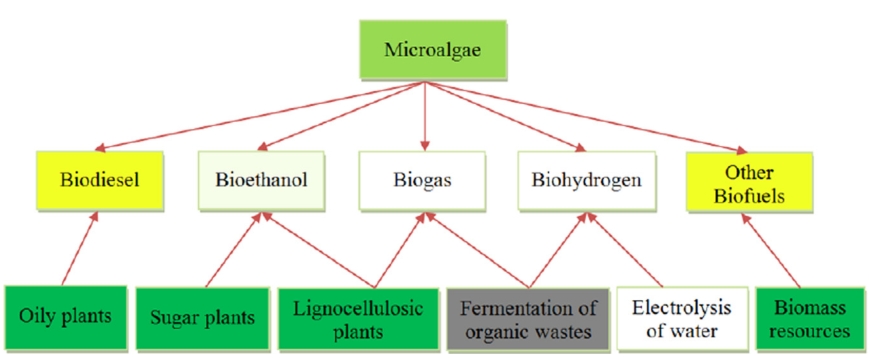

Fig. 1. Flexible biofuels production from microalgae for sustainable development.

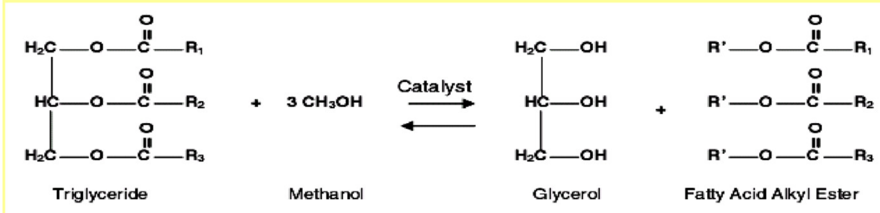

Fig. 2. Transesterification of oil to biodiesel (R_{1-3} are long chain hydrocarbon groups) [70].

as well as dairy and cattle manures involved in a few studies [81]. The following aspects have got a lot of focus: the composition ratios of cellulose, hemicellulose and lignin, efficient enzyme development and methods to release sugar components for fermentation [82]. Lignin is very difficult to be degraded biologically and cannot be fermented directly [83], and thus pretreatment is necessary. Usually, pre-treatment, such as steam explosion, is energy intensive to break down the complex structure of cell wall which is rich in lignin and cellulose. And thus, until now no commercial ethanol plants appear at a large scale due to the high costs.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow 2nCH_3CH_2OH + 2nCO_2 \quad (1)$$

Apart from biodiesel production, microalgae are also a fermentation feedstock to produce bioethanol, although there is limited literature related to microalgal bioethanol. Carbohydrates in microalgae can be hydrolyzed to sugars [84] and then fermented to bioethanol by yeast. The final product, ethanol, can be attained after distillation process. Compared to woody biomass, microalgae have some special properties, which are beneficial to bioethanol fermentation. First, microalgal cell walls are largely made up of polysaccharides with low/no percentage of lignin and hemicelluloses, which can favor the hydrolysis of cell walls to sugars [83,85]. Thus, it can accelerate the bioethanol production process, because no chemical and enzymatic pre-treatment is mandatory. Nevertheless, the physical pre-treatment, such as extrusion and supercritical CO_2, is still needed to break down the cell wall, release the carbohydrates and finally convert them to sugars [86,87]. Second, the composition of microalgae is generally much more identical and consistent than that of woody biomass, since they do not have roots, stems and leaves, which is advantageous to pretreatment [85]. Finally, microalgae, such as Chlorella, Dunaliella, Chlamydomonas, Scenedesmus and Spirulina, contain more than 50% (dry weight) of starch and glycogen, which are useful ingredients for bioethanol production [86]. Bioethanol production yields from different microalgae species are listed in Table 3.

It is possible to simultaneously produce biodiesel and bioethanol from microalgae. The green microalgae *Chlorococum* sp. with pre-extracted lipids produces 60% higher ethanol concentrations than those that remain as dried intact cells [83], since during lipid extraction supercritical CO_2 can break down microalgae cell wall, resulting in the simultaneous release of carbohydrates ready for bioethanol production. In addition, genetic modification technologies for selected strains have also been used in an attempt to optimize microalgal bioethanol production. Deng and Coleman [96] genetically modified green algae to produce ethanol from sunlight and CO_2 by introducing new genes into cyanobacterium. Similarly, the Algenol Company is designing and developing a strain of GM cyanobacteria that are capable of producing ethanol in Mexico [42].

Bioethanol production from microalgae is still in the preliminary research phase, where further research about its advantages and disadvantages needs to be concluded. More researches, including the genetic engineering of selected strains and the development of new bioreactors for effective production, are still required to investigate and analyze the technologies. Therefore, its future development deserves more attention and expectation.

Table 3
Bioethanol production yields from different microalgae species.

Feedstock	Pretreatment	Yield (g ethanol/g substrate)	Citation
Kappaphycus alvarezii	Sulfuric acid	0.457	[88]
Gracilaria verrucosa	Sulfuric acid and enzymatic	0.430	[89]
Saccharomyces cerevisiae	Enzymatic	0.259	[90]
Chlorococum humicolo	Sulfuric acid	0.520	[91]
Chlorella vulgaris	Acid and enzymatic	0.400	[92]
Chlorococum sp.	Supercritical CO_2	0.383	[83]
Chlorococum infusionum	Alkaline	0.260	[93]
Gelidium amansii	Sulfuric acid	0.888	[94]
Chlamydomonas reinhardtii	Enzymatic	0.240	[95]

3.3. Biogas

Biogas generation is a long-established technology. Previous studies have indicated that anaerobic digestion is technically viable [42] and widely used for the treatment of all kinds of wastes [97]. Biogas achieved by anaerobic digestion mainly contains methane and carbon dioxide. Biomass used for anaerobic digestion can be obtained from (1) terrestrial sources, including mechanically sorted and hand-sorted municipal solid wastes, various types of fruit and vegetable solid wastes, leaves, grass, wood and weed, and (2) aquatic sources, including both marine and freshwater biomass, such as seaweed and sea-grass [98]. The technology for anaerobic digestion of waste biomass exists already and is well established and developed [99]. So far, the yield of biogas achieved has varied from 0.15 to 0.65 m^3 per kg of dry biomass [100]. The main disadvantages of the utilization of terrestrial sources are their availability and competition with farmlands for food crops.

$$C_aH_bO_cN_d + \frac{1}{4}(4a - b - 2c + 3d)H_2O \rightarrow \frac{1}{8}(4a + b - 2c - 3d)CH_4 + \frac{1}{8}(4a - b + 2c + 3d)CO_2 + dNH_3 \quad (2)$$

Apart from biomass mentioned above, the microalgae or their residues after lipid extraction have a huge potential for biogas production as well, since their lipid, starch and protein content can respectively come up to 70%, 50% and 50% without lignin [98]. According to the research by Chisti [100], after 30% oil content has been removed from algae, algal residuals can provide at least 9360 MJ of energy per metric ton, a substantial amount of energy, which can make microalgal biodiesel production more sustainable. The idea of anaerobic digestion of microalgae was firstly mentioned by Golueke et al. [101] and well researched by Park and Li

Table 4
Biogas production yields from different microalgae species.

Feedstock	Yield[a]	Methane content (%)	Citation
Scenedesmus obliquus	0.240 L CH_4 g^{-1} VS		[98]
Phaeodactylum tricornutum	0.360 L CH_4 g^{-1} VS		[98]
Chlorella vulgaris	0.375 L g^{-1} S	–	[106]
Blue algae from Taihu Lake, China	0.190 L g^{-1} VS	36.7	[107]
Chlamydomonas reinhardtii	0.587 L g^{-1} VS	–	[104]
Macrocystis pyrifera	0.180 L g^{-1} S	65.0	[108]
Durvillea antarctica	0.180 L g^{-1} S	65.0	[108]
Chroococcus sp.	0.401–0.487 L CH_4 g^{-1} VS	52.0–54.9%	[109]

[a] L g^{-1} VS means liter per gram of volatile solids, while L g^{-1} S represents liter per gram of solids.

[102] and Zhong et al. [103]. During anaerobic digestion, biogas can be produced and nutrients (N, P and K) can be greatly recycled towards the microalgae cultivation systems. The efficiency of biogas production is strongly dependent on the species and pretreatment [104]. Practical biogas yield is about 0.5 L per dry weight [105]. Biogas production yields from different microalgae species are listed in Table 4.

The production of biogas from microalgae biomass can have technoeconomic potential by using high-rate anaerobic digesters [105], such as upflow anaerobic sludge blanket (UASB), anaerobic filter (AF) and anaerobic membrane bioreactor (AnMBR), since costs can be reduced due to the low requirement on biomass dewatering [98]. Research by Stucki et al. [110] showed that microalgae (*Spirulina platensis*) could be completely gasified to a methane-rich gas in supercritical water using ruthenium catalysts and 60–70% of the heating value contained in the microalgal biomass would be recovered as methane. Vergara-Fernándeza et al. [108] achieved 70% of the total biogas with the similar yield of 180.4 mL g^{-1} dry weight day^{-1} and a methane concentration around 65% when using an upflow anaerobic filter to evaluate anaerobic digestion of two marine algae species: *Macrocystis pyrifera* and *Durvillea Antarctica*.

The generation of biogas from microalgae might also have an important role to play in water ecosystem rehabilitation, since removing and collecting the harmful algal blooms in lakes, ponds, rivers, reservoirs or seas can reduce the potential risks, for example, the production of toxic secondary metabolites [107]. Nonetheless, the processes generating biogas from microalgae are still at pre-commercial stages for the time being, since the highest energy demand comes from heating the digesters and it requires more land surfaces and facilities to generate 1 MJ of methane-based biofuel than 1 MJ of algal biodiesel [106]. Moreover, the retention time required for considerable degree of digestibility is 20–30 days on average [105], which increases the costs for large scale establishment. Pretreatments, such as thermic or sonic treatments, can solve the problems, since they can decrease the resistance of the cell wall to increase the reaction speed and the total biodegradability of particulate matters. Another option is to increase the C/N and hence to reach a more favorable range for anaerobic digestion. Zhong et al. [103] suggested the optimal C/N ratio for co-digestion of algae with corn straw is 20/1. Park and Li [102] co-digested algae biomass residue with lipid-rich fat, oil and grease waste and achieved methane yield at 0.54 L CH_4 g^{-1} VS day^{-1}. In addition, it was found that when algae *Chlorella* sp. was co-digested with waste activated sludge, not only biogas yield of microalgae was improved but the gas phase was reached more quickly [111].

3.4. Biohydrogen

Biohydrogen is a promising clean biofuel type in the future, since it can be used in a fuel cell with only water as the exhaust product without any pollutant (such as, SO_x, NO_x, etc.) emissions. Traditionally, hydrogen is produced by the process of steam reformation of fossil fuels, such as crude oil and coal. Although large-scale electrolysis of water is also possible, it costs more energy than can be generated from the hydrogen by this method. These two chemical methods are not cost-effecitve due to the high energy requirements [112]. It is also reported that several bacteria, such as purple non-sulfur bacteria [113,114], can use a wider range of organic substrates (such as food wastes, agricultural residues and wastewaters) and light to produce hydrogen.

Microalgae can also directly use sunlight and water to generate biohydrogen in the absence of oxygen in a closed culture system [115]. During photolysis, microalgae can split two water molecules via photosynthesis to form one oxygen molecule and four hydrogen ions which can be converted into two hydrogen molecules by hydrogenase enzyme [112,116,117]. Some microalgae, such as *Scenedesmus obliquus* [118], *Playtmonas subcordiformis* [119], *Scenedesmus* sp. [120] and *Chlamydomonas reinhardtii* [121], have been detected to have substantial hydrogenase activity in biohydrogen formation. For example, enzyme activity of *C. reindhartii* reported by Winkler et al. [120] is robust, arriving at 200 nmol μg^{-1} Chla h^{-1}. The generated oxygen has strong inhibition effect on hydrogenase enzyme, which can be relieved by the production of microalgae under sulfur deprivation for 2–3 days to lead to the anaerobic conditions [120–122] for biohydrogen production.

Wecker et al. [123] successfully designed a new biosensor to grow *Rhodobacter capsulatus* and *Chlamydomonas reinhardtii* in the dark to produce efficient H_2, while Chatzitakis et al. [117] employed a photoelectrocatalytic-enzymatic hybrid system for simultaneous hydrogen production and organic pollutant reduction. Lee et al. [124] attained a total of 444 mL of bio-hydrogen produced from 10 g l^{-1} of dry algae in a 100 ml of culture fluid for 62 h when marine brown algae (*Laminaria japonica*) were fed under dark fermentation conditions. However, it is not possible to produce the theoretical maximum of 20 g H_2 m^{-2} day^{-1} in practice [85], making bulk-scale hydrogen production by algae unfeasible at this moment. One major limiting factor for the full-scale production of biohydrogen is the absence of a large-scale sustainable production method to improve hydrogenase activity and the lack of efficient microalgae to yield biohydrogen. Therefore, in an effort to thrive on the profitable and renewable hydrogen production, it requires more researches on organism species, the optimized operation conditions and genetic modification to optimize the use of solar energy and the activity of hydrogenase enzyme. Hydrogen production from microalgae is still some way from commercial viability, but continued progress will testify its ultimate potential.

3.5. Other biofuels types

Except biodiesel, bioethanol, biogas and biohydrogen, microalgae can also be converted into biobutanol, bio-oil, syngas, jet fuel, etc. via appropriate conversion methods, including thermochemical, chemical and biochemical conversion processes. Butanol can replace gasoline in most gasoline engines without any modification, and when it is

blended with gasoline, it can provide better performance and corrosion resistance than ethanol. Ellis et al. [125] reported that the fermentation of acid/base pretreated microalgae (*Clostridium saccharoperbutylacetonicum*) could produce 0.201 g butanol g^{-1} substrate, as compared with 0.208 g butanol g^{-1} substrate from pretreated algae supplemented with 1% glucose and 0.249 g butanol g^{-1} substrate from pretreated algae catalyzed by xylanase and cellulase enzymes. Bio-oil is synthetic liquid fuel that is extracted by processing biomass in a reactor at a high temperature in the absence of oxygen. Bio-oil can be directly used in engines or in a blend [126]. A range of microalgae have shown the potential of producing bio-oil through pyrolysis or thermochemical liquefaction of microalgae [126–128]. Syngas is a gas mixture that contains very low concentrations of hydrocarbons and higher concentrations of CO and H_2 generated by oxygen-gasification processes [129]. Syngas is a versatile feedstock and can be converted into diesel fuel by Fisher–Tropsch synthesis process [130], making it possible to integrate an algal feedstock into an existing thermochemical infrastructure. Microalgae-derived jet fuel has also received attention [130]. Jet fuel blends (derived from a variety of oil-rich feedstock, including algae) have shown to be compatible for use in selected commercial demonstration jet test flights [131].

4. Combined production of bioethanol and biogas to make biodiesel sustainable

During biodiesel production, large amounts of residuals (containing carbohydrates, proteins, fats, etc.) will be generated simultaneously, accounting for about 65% of the microalgae biomass [132]. In an attempt to make microalgal biodiesel sustainable, it is necessary to use every component of the algal biomass. As explained already, lipids can be refined into biodiesel, while carbohydrates can be converted to ethanol, and proteins, fats and the leftovers of lipids and carbohydrates after ethanol conversion can be used to produce into methane. Thus, a combined biorefinery approach (Fig. 3), where multiple biofuels are produced, has been put forward by Zhu [133].

As shown in Eq. (1), the theoretical yields are 0.5 kg ethanol and 0.49 kg CO_2, per kg of carbon sugar, i.e. glucose. As shown in Eq. (2), the methane yield (liter of CH_4 per gram of solids) can thus be calculated as: $V=[V_m\times(4a+b-2c-3d)]/[8(12a+b+16c+14d)]$, where V_m is the normal molar volume of CH_4. On the basis of this calculation, Angelidaki and Sanders [134] obtained the according specific methane yield at 0.5 L/g-S. According to our quantitative experiments, only about one-third to one forth of lipids could be converted into biodiesel, since some lipid types, such as chlorophyll, glycolipid and phospholipid are not the efficient ingredients for biodiesel conversion [23–25]. Thus, it is estimated that 30% of lipids can be converted into biodiesel. Based on the above analysis and assumption, Table 5 lists the theoretical biodiesel, ethanol and methane yield potential in the biofuels production chain [133].

The combined biorefinery and recovery of biodiesel, bioethanol and biogas can significantly lead to an increase in the energetic productivity of the microalgal biomass from a sustainability perspective (Table 5). Therefore, the conversion of bioethanol and biogas from algal residues is a key measure to keep both energetic and economic aspects in a balance manner, especially for

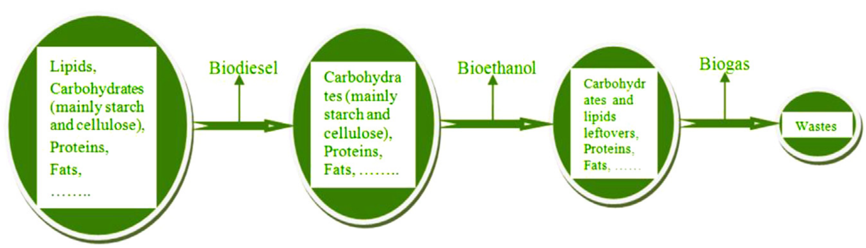

Fig. 3. A proposal for the combined microalgal biofuels production chain.

Table 5
Gross composition of several microalgae species expressed on a % dry matter basis [135,136] and theoretical biodiesel, ethanol and methane yield potential [133] on the basis of lipid, carbohydrate, and protein contents, respectively.

Microalgae	Lipid (%)	Carbohydrates (%)	Protein (%)	Biodiesel (g/kg-S)	Ethanol (g/kg-S)	Methane (L/g-S)
Freshwater microalgae species						
Anabaena cylindrica	4–7	25–30	43–56	12–21	125–150	0.22–0.28
Aphanizomenon flosaquae	3	23	62	9	115	0.31
Arthrospira maxima	6–7	13–16	60–71	18–21	65–80	0.30–0.36
Chlamydomonas rheinhardii	21	17	48	63	85	0.24
Chlorella pyrenoidosa	2	26	57	6	130	0.29
Chlorella vulgaris	14–22	12–17	51–58	42–66	60–85	0.26–0.29
Chlorella zofingiensis	26–46	25–28	11–20	78–138	125–140	0.06–0.10
Euglena gracilis	14–20	14–18	39–61	42–60	70–90	0.20–0.31
Scenedesmus dimorphus	16–40	21–52	8–18	48–120	105–260	0.04–0.09
Scenedesmus obliquus	12–14	10–17	50–56	36–42	50–85	0.25–0.28
Scenedesmus quadricauda	1.9	–	47	5.7	–	0.24
Spirogyra sp.	11–21	33–64	6–20	33–63	165–320	0.03–0.10
Spirulina maxima	6–7	13–16	60–71	18–21		
Spirulina platensis	4–9	8–14	46–63	12–27	40–70	0.23–0.32
Marine microalgae species						
Dunaliella bioculata	8	4	49	24	20	0.25
Dunaliella salina	6	32	57	18	160	0.29
Porphyridium cruentum	9–14	40–57	40–57	27–42	200–285	0.20–0.29
Prymnesium parvum	22–38	25–33	28–45	66–114	125–165	0.14–0.23
Synechococcus sp.	11	15	63	33	75	0.32
Tetraselmis maculata	3	15	52	9	75	0.26

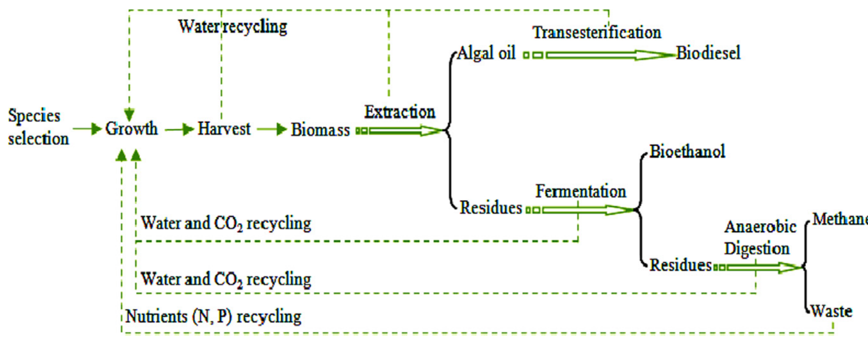

Fig. 4. Mass flow and recycling system for the microalgae biorefinery chain [133].

the low-lipid-content species [133]. This bio-ethanol and/or biogas can provide the primary energy source for the whole biorefinery process. In case this primary energy source was surplus, it could be transferred and sold, for instance, to grid, to further improve the economics of the combined system [100].

After biofuels production, organic N and P in algal wastes can be mineralized to a flux of ammonium and phosphate which can be either be recycled as a substrate for microalgae growth [26] or could be sold as soil conditioners and fertilizers [133]. The water used and CO_2 formed in these two processes can be recycled to algae growing systems (Fig. 4). And thus, from a sustainability viewpoint the processing water and nutrients (N and P) can be recycled greatly and the recovery of bioethanol and biogas can potentially result in an energetic balance of the microalgae to biofuels process, which can improve the economics of algal biorefinery approach.

5. Conclusions

Industrial reactors currently being pursued for microalgae culture at a relevant scale are open ponds, photobioreactors and fermenters, all of which are technically feasible. The three types of culture vessels have their own advantages and disadvantages, the selection of which as the culture facility can be decided according to the real conditions in practice. Microalgae, as a versatile feedstock, have broad bioenergy potential, since they can flexibly produce multiple biofuels, such as biodiesel, bioethanol, biogas and biohydrogen, via different conversion methods. The integrated algal biofuels production, where biodiesel, bioethanol and biogas are continuously produced, can potentially result in the improvement of the economics of algal biorefinery approach, and in this approach processing water and nutrients (N and P) can be recycled greatly to the microalgae cultivation systems.

6. Perspectives

Undoubtedly, microalgae-based biofuels have a paramount role to play in combating energy shortage, global warming and climate changes. Nevertheless, research for the production of biofuels from microalgae is in the beginning stages in light of the research advances still required [67], and thus we are still some way from realizing the potential to produce commercially viable microalgal biofuels at a large scale. The main hurdle to be solved is the cost gap, since the technologies (including cultivation, harvesting and conversion) accessible today cannot make microalgal biofuels production economical. The cost of the infrastructure facilities and the energy required for microalgae cultivation and harvesting are high [1,137,138], mainly due to the drying operation of the biomass, which has been reported to consume around 69% of the input energy [20]. Given this, there is a substantial demand for more researches to study and improve their economics in an effort to support a sustainable biofuel industry.

First, genetic engineering technology can contribute a lot to an increase of the productivity of microalgae [133,139], and thus improve the economics of production of algal biofuels. The basic understanding of the biology of microalgae can facilitate gene cloning and manipulation, allowing the power of genomics, proteomics and metabolomics technologies to be applied in practice [140]. Scientists have fully interpreted and sequenced the genomes of a number of microalgae, such as *Heterosigma akashiwo* and *Chattonella marina* var. *marina* [141], and identified the relevant bioenergy genes and pathways in microalgae, followed by successfully engineering some strains [142]. Genetic modification in molecular level can be used to increase the photosynthetic efficiency and productivity by enhancing biomass growth rate and oil content, and also to improve temperature toleration ability of microalgae in an attempt to decrease the requirement for costly cooling [139]. Relative pioneering achievements, each with an opportunity for commercial application [140], include the improved methods for trophic conversion, augmented lipid biosynthesis, engineered light-harvesting antennae and recombinant protein expression. For example, Mussgnug et al. [143] successfully apply RNAi technology to down-regulate the entire gene family of light-harvesting antenna complexes to improve their photosynthetic efficiency. Another current example is that the Algenol Company is developing a strain of GM cyanobacteria to produce bioethanol [42].

Second, metabolic engineering technology also has a critical role to play in species development to obtain efficient strains to produce biofuels. Metabolic engineering techniques, including carbon partitioning and microalgae engineering without photoinhibition or with a higher inhibition light threshold, can greatly improve productivity [100]. Zaslavskaia et al. [144] found that the photoautotrophic diatom *Phaeodactylum tricornutum* became able to grow heterotrophically in darkness after the authors inserted glucose transporter genes from Chlorella into this species. Metabolic engineering allows direct control over the organism's cellular machinery by mutagenesis techniques or the introduction of transgenes [140]. Metabolic engineering techniques are environmentally sensitive, since different environmental conditions can lead to complicated metabolic pathways of a cell. Stress conditions can induce spontaneous changes in metabolism in cultivated algae strains, enriching biomass with the targeted metabolite [42]. A natural mechanism is that under nitrogen depletion or starvation microalgae can alter lipid metabolism, showing that the rate of lipid synthesis remains higher [140]. It is reported that more than 2300 algae species have been collected, which will provide increasingly useful resources for the application of post-genomic technologies [145].

Third, the economics of microalgal biofuels production can be improved by using seawater, wastewaters, fermented liquid

(biogas liquor), manure, and other sources from agriculture as nutrient source [146–149] and combustion gas like flue gas as CO_2 source [139,150] to mitigate environmental impacts. Successful implementation of this strategy can allow the minimization of the use of freshwater and fertilizer and the sequestration of CO_2. Using wastewaters as nutrients, microalgae can also degrade the inorganic and organic matters in wastewaters mainly by physical processes and microbial activity [151], thus providing a new method for wastewater treatment. Wang and Lan [152] achieved the final algal biomass concentration at 2.1 g dry cell weight L^{-1} with a relative biomass productivity of 233.3 mg day^{-1} when growing *Neochloris oleoabundans* in municipal wastewater effluents containing 70 mg N L^{-1}. Microalgae can tolerate a high CO_2 content in feeding air streams, and on average, producing 100 t of mciroalgal biomass can fix 183 t of CO_2 [139]. De Morais and Costa [153] cultivated *Spirulina* sp. and *Scenedesmus obliquus* in a three-stage serial tubular photobioreactor and obtained daily CO_2 biofixation of 0.413 and 0.260 g L^{-1}, respectively. A higher CO_2 mitigation rate between $50.1 \pm 6.5\%$ on cloudy days and $82.3 \pm 12.5\%$ on sunny days was achieved by Vunjak-Novakovic et al. [154], who grew *Dunaliella parva* and *Dunaliella tertiolecta* in air-lift reactors supplied with flue gases from a power plant.

Forth, extensive studies have been conducted to explore the feasibility of the high-value co-product/byproduct strategy. Microalgae contain abundant nutrients and a variety of inorganic and complex organic molecules, which can be simultaneously converted into and used as pharmaceutical materials [155], health foods and natural pigments [32,156]. Some well-studied examples include pharmaceuticals, polysaccharides, stable isotopes, polyunsaturated fatty acids, anti-oxidants, glycerol, biodegradable polymers, cosmetics and coloring substances [129]. *Muriellopsis* sp. has been discovered to have the ability to accumulate high levels of carotenoids, such as lutein, which has a good effect on the prevention and treatment of degenerative diseases [157]. Nilles [158] reported that more than 400,000 t of glycerol could be simultaneously co-produced during the extraction of 1 billion gallons of algal biodiesel. To summarize, the integration of biorefinery approach with high-value co-product/byproduct strategy is expected to enhance the economics of algal biofuels production in a sustained manner.

Finally, several technological improvements ranging from pretreatment to conversion processes can provide a platform as well to drive more relevant and problem-solving research activities for further development. The efficiency of conversion is strongly dependent on, apart from the species, the pretreatment [104,159]. Pretreatments, such as thermic or sonic treatments, which can decrease the resistance of the cell wall to increase the reaction speed and the total biodegradability, are still needed to be optimized under different environmental conditions. For instance, enzymatic hydrolysis from the complex algal cell wall can be used to unlock valuable biochemical molecules, thus reducing energy costs, since there is no need to dewater the algal biomass during harvest [138]. Extensive studies on efficient biomass conversion solutions, such as supercritical extraction/transesterification, vacuum pyrolysis and transesterification assisted with ultrasonication or microwave, also require to be optimized and are yet to be discovered to be effective [32,87].

Although there are some obstacles on the large-scale commercial production of biofuels at present, microalgae are still the most promising and best feedstock available for the biofuels. Achieving the ultimate potential to produce the economic biofuels from microalgae is of strategic importance to sustainable development. It is urgent to bring evident breakthroughs to produce sustainable biofuels for future development. Biotechnology advances including genetic and metabolic engineering, high crude oil prices and high carbon values can make microalgal biofuels outcompete all other fuels, and well-funded R&D researches on biotechnology and policy support can help realize this scenario.

Acknowledgment

This work was partially funded by the National Basic Research Program of China (Grant no. 2011CB200905). This work was also partially supported by the Fortum Foundation and the South Ostrobothnia Regional Fund of the Finnish Cultural Foundation in Finland. We are grateful for this support. We would also like to thank the people who have participated in the discussion on the aspects of algal biofuels for sustainable development. The authors would also like to thank the two anonymous reviewers for their helpful comments and suggestions that greatly improved the manuscript.

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